iGEM 2016: Pittsburgh
Week 9 Lab Notebook

Monday, July 18

Lab meeting

Dilution series of cell-free reactions with lacZ Collins plasmids (Claire)

25 ng switch and trigger plasmids

20 reactions

Master (22 rxns): 44 uL Solution A
33 uL Solution B
5.5 uL RNAse inhibitor
5.5 uL substrate

Into 2 tubes, each 44 uL

D: 3.59 uL switch, 1.69 uL trigger, 5.72 uL water

G: 2.88 uL switch, 1.85 uL trigger, 6.27 uL water

Pipette each tube into 10 wells, 5 uL each

Dilute to 10, 15, 20, or 25 uL with water

Row G: D switch

Row H: G switch

Columns 1-2: 5 uL

Columns 3-4: 10 uL

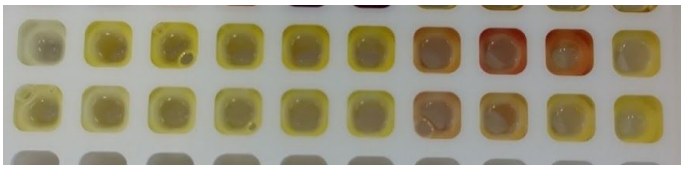
Columns 5-6: 15 uL

Columns 7-8: 20 uL

Columns 9-10: 15 uL

Run time course for 2 hours, readings every minute with incubation of 37 degC without lid

Dilute 5 uL reaction to 10 uL and read again



Picture from plate bottom (so columns reversed)

Barely any purple, except for 10 uL reactions

Place in incubator

Double digest of colony 3 (PT7-RBS-amilCP) (Maya)

40 uL reaction with 5 ug DNA to gel extract

2 uL EcoRI

2 uL SpeI

4 uL Buffer

27 uL DNA

5 uL H2O

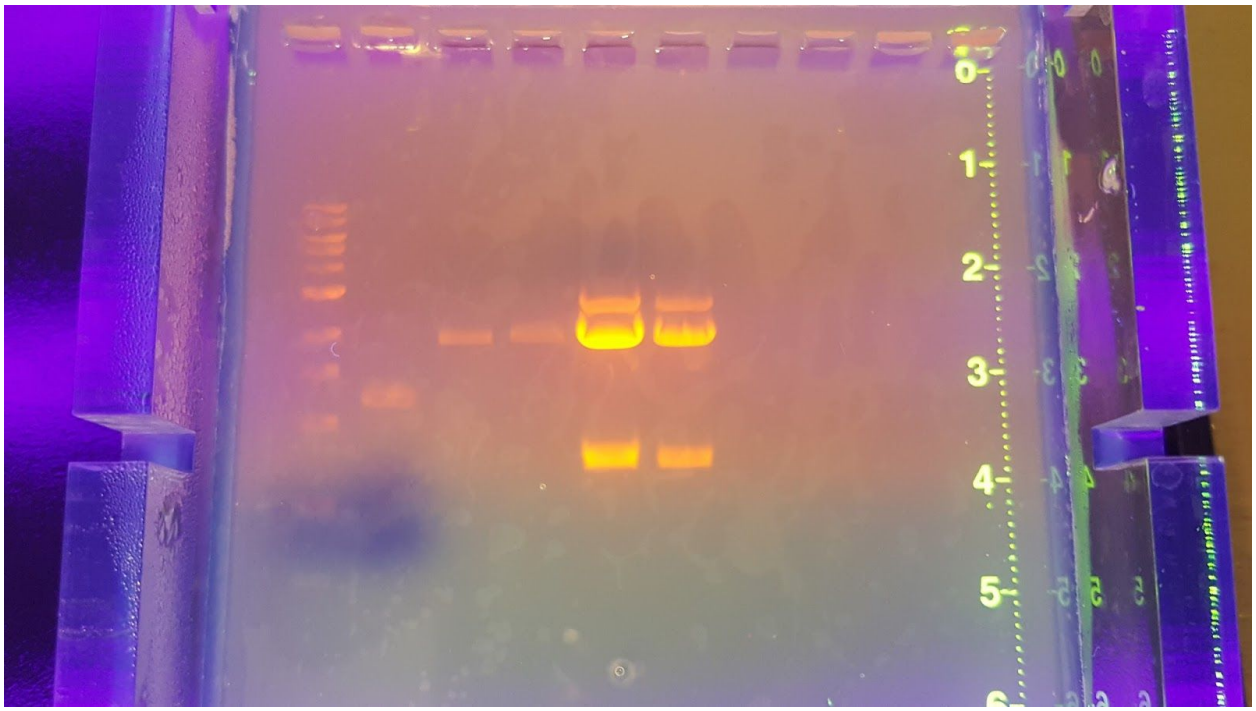
3:1 and 7:1 ligation check (PT7-amilCP) (Maya)

10 uL digest with 100 ng DNA

Both:	0.5 uL EcoRI
	0.5 uL PstI
	1 uL Buffer
3:1:	0.528 uL DNA
	7.47 uL H2O
7:1:	0.501 uL DNA
	7.50 uL H2O

Gel (Both double digest and ligation check) (Maya)

Lane 1: Ladder	Lane 4: 7:1
Lane 2: Control (Uncut T7)	Lane 5: Colony 3
Lane 3: 3:1	Lane 6: Colony 3



More liquid cultures of 3:1 and 7:1 as neither were successful colonies (Maya)

Gel extracted colony 3 (3.9 ng/uL) (Maya)

Reach out for fundraising

Tuesday, July 19

Miniprep liquid cultures from previous day (Maya)

Ligation check digest for PT7-RBS-amilCP for 3:1 and 7:1 liquid cultures (Maya/Maddie)

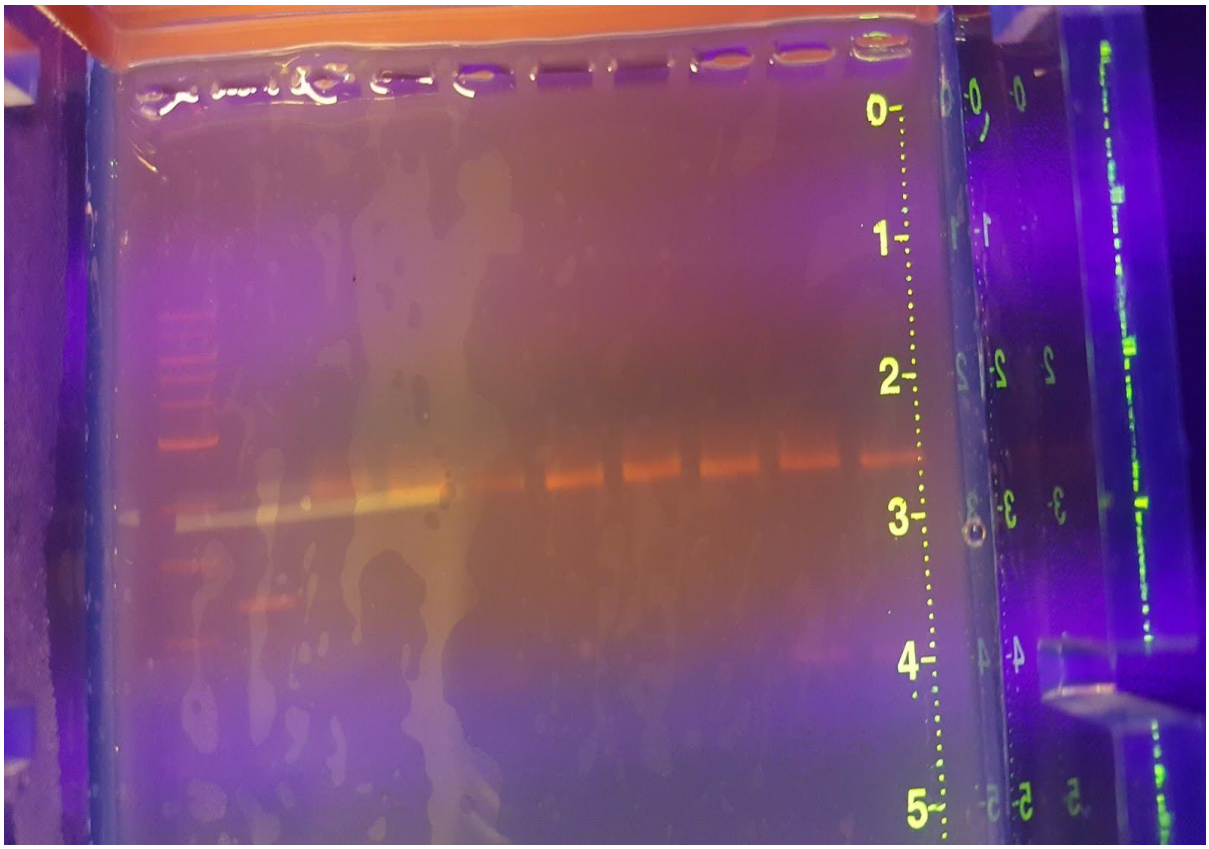
100 ng, 10 uL reactions

For all:		0.5 uL EcoRI
		0.5 uL PstI
		1.0 uL Buffer
3:1	1	0.831 uL DNA
		7.17 uL H2O

	2	0.510 uL DNA
		7.49 uL H2O
	3	0.416 uL DNA
		7.58 uL H2O
7:1	1	0.617 ul DNA
		7.38 ul H2O
	2	0.842 ul DNA
		7.16 ul H2O
	3	0.638 ul DNA
		7.36 ul H2O
	4	0.483 ul DNA
		7.52 ul H2O
	5	0.504 ul DNA
		7.50 ul H2O

Gel to check for PT7-amilCP (Maya/Maddie)

Lane 1: Ladder
Lane 2: Control (Uncut T7)
Lane 3: 3:1-1
Lane 4: 3:1-2
Lane 5: 3:1-3
Lane 6: 7:1-1
Lane 7: 7:1-2
Lane 8: 7:1-3
Lane 9: 7:1-4 (Successful)
Lane 10: 7:1-5 (Successful)

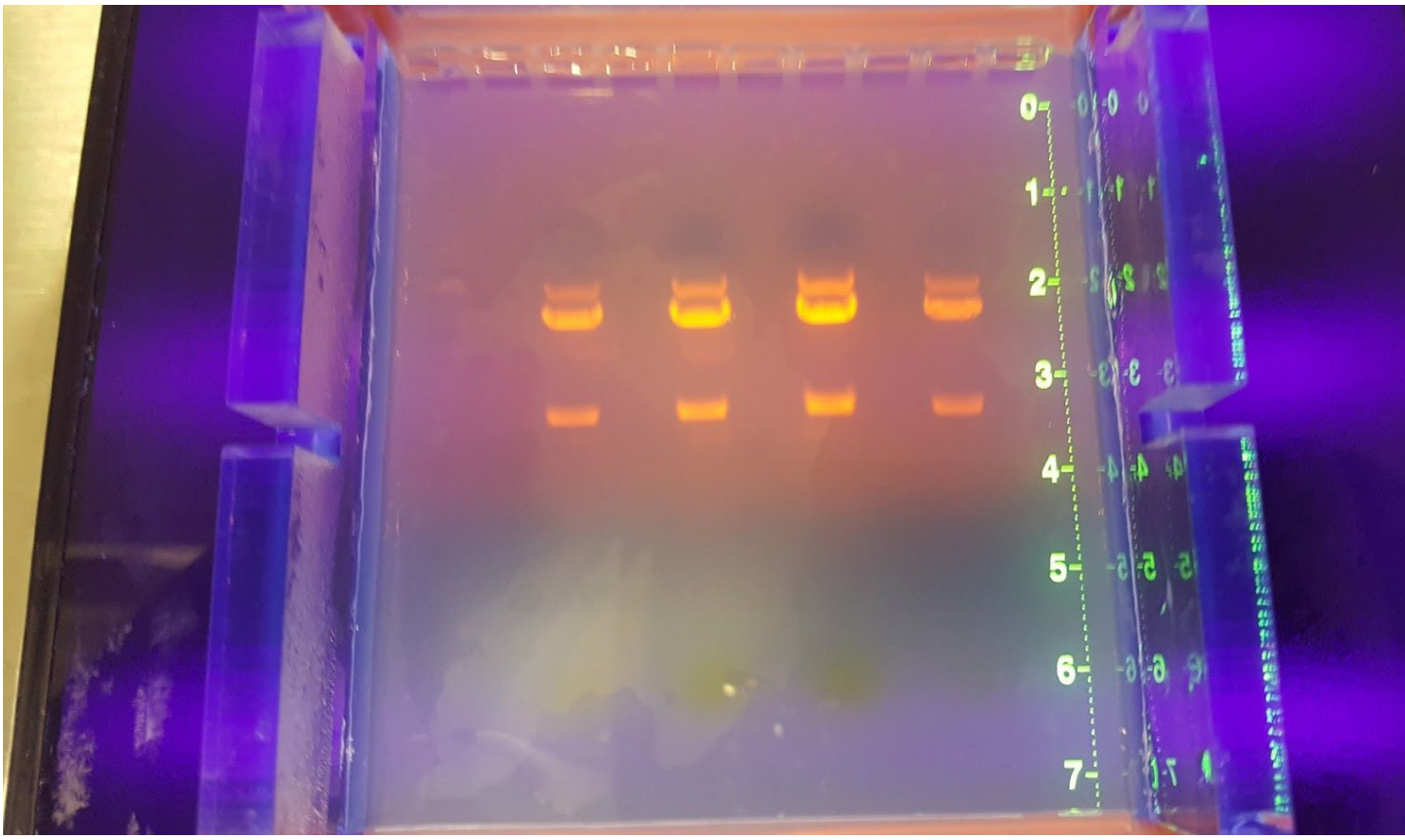


Digest 7:1 4 and 5 to gel extract PT7-amilCP (Maya/Maddie)

5 ug DNA in a 40 uL reaction
45 min at 37C
20 min at 65C
For both: 2 uL EcoRI
2 uL SpeI
4 uL Buffer
4 (207.2 ng/uL): 24.13 uL DNA
7.87 uL H2O
5 (198.3 ng/uL): 25.2 uL DNA
6.8 uL H2O

Gel for 7:1 4 and 5 (20 uL per well of digest) (Maya/Maddie)

Lane 1: Ladder
Lane 2: Control (Uncut T7)
Lane 3: 7:1-4
Lane 4: blank
Lane 5: 7:1-4
Lane 6: blank
Lane 7: 7:1-5
Lane 8: blank
Lane 9: 7:1-5
Lane 10: blank



Ligation of PT7-RBS-amilCP with Terminator (Maya/Maddie)

Plasmid: Terminator (16.8 ng/uL)
Insert: PT7-RBS-amilCP

50 ng plasmid
For all: 2 uL Buffer
1 uL T4 DNA Ligase
Control (no insert)
2.98 uL plasmid
0 uL insert
14.02 uL H2O
3:1 (insert:plasmid)
2.98 uL plasmid
2.78 insert (54.2 ng/uL)
11.24 H2O
7:1 (insert:plasmid)
2.98 uL plasmid
6.25 uL insert (56.0 ng/uL)
7.77 uL H2O

Transformation (Maya/Maddie)
What we believe to be LacZ from previous year's stock
RBS-T3 from iGEM kit

Cell-free dilution repeat with 50 ng switch DNA, 1 uM trigger DNA (Claire)

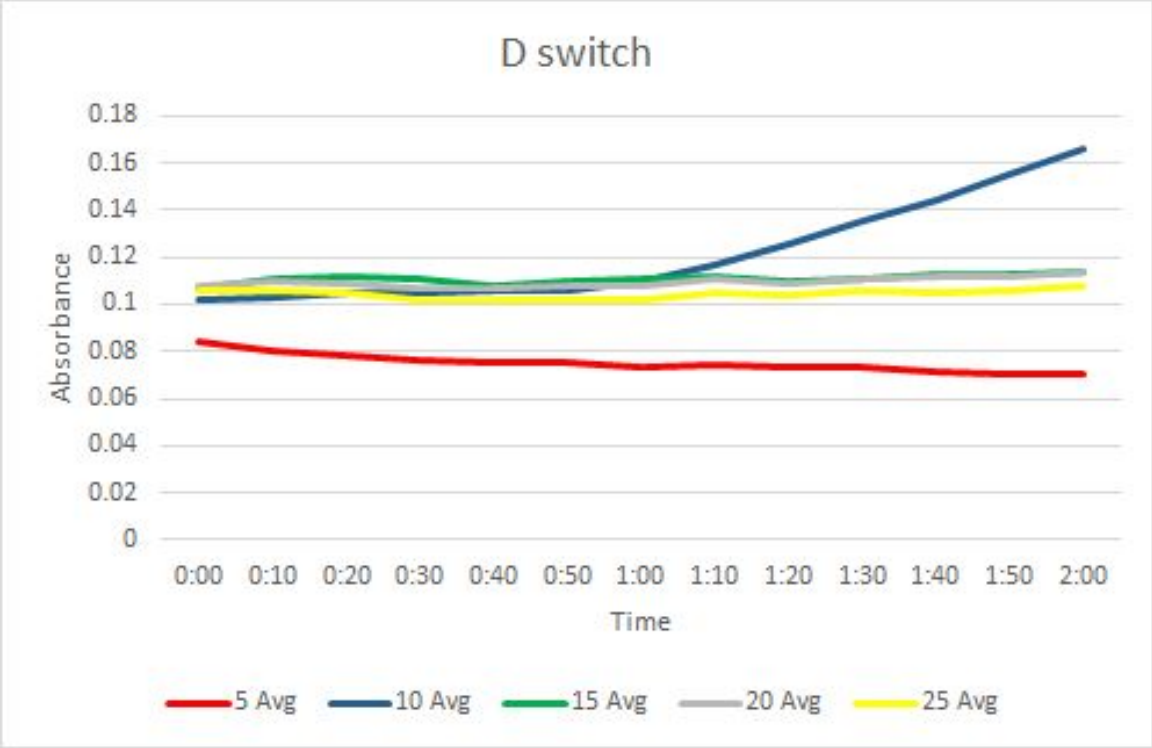
D:	Solution A	2 uL		24 uL
	Solution B	1.5 uL		18 uL
	RNAse inhibitor	0.25 uL	x 12 reactions =	3 uL
	Substrate	0.25 uL		3 uL
	Switch	0.66 uL		7.92 uL
	Trigger	0.34 uL at 2.5 uM		4.08 uL

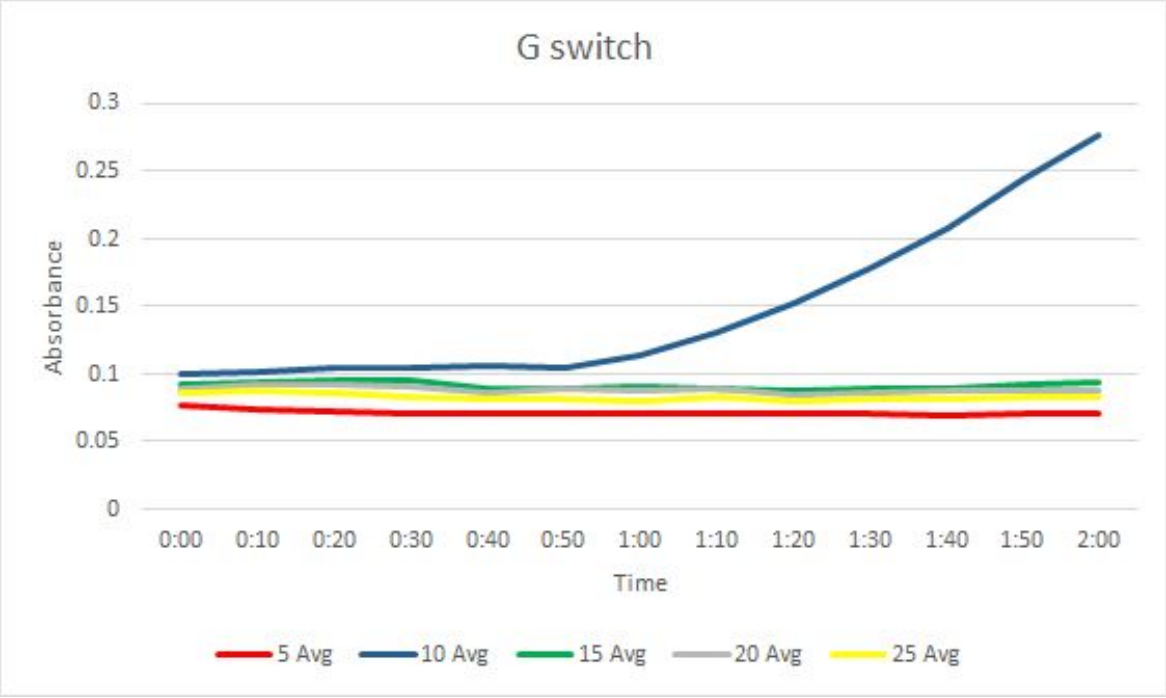
G:	Solution A	2 uL		22 uL
	Solution B	1.5 uL		16.5 uL
	RNAse inhibitor	0.25 uL		2.75 uL
	Substrate	0.25 uL	x 11 reactions =	2.75 uL
	Switch	0.59 uL		6.49 uL
	Trigger	0.34 uL at 2.5 uM		3.74 uL
	Water	0.07 uL		0.77 uL

Master:	Solution A	46 uL
	Solution B	34.5 uL
	RNAse inhibitor	5.75 uL
	Substrate	5.75 uL

Pipette 5 uL reaction into each well, dilute to appropriate volume
Time course with lid; leftover reactions in heat block
Barely purple after 2 hours, back in incubator
Row G: D switch
Row H: G switch

Columns 11-12: 5 uL
Columns 13-14: 10 uL
Columns 15-16: 15 uL
Columns 17-18: 20 uL
Columns 19-20: 15 uL
Column 21: leftover reaction





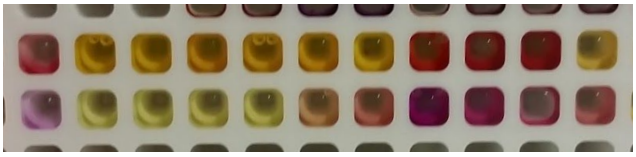


Plate from underneath (columns go right to left)

Dilution 1 from yesterday developed more intense color, especially 15 uL

Run gel with quenched DNAzyme reactions (Praneeth)

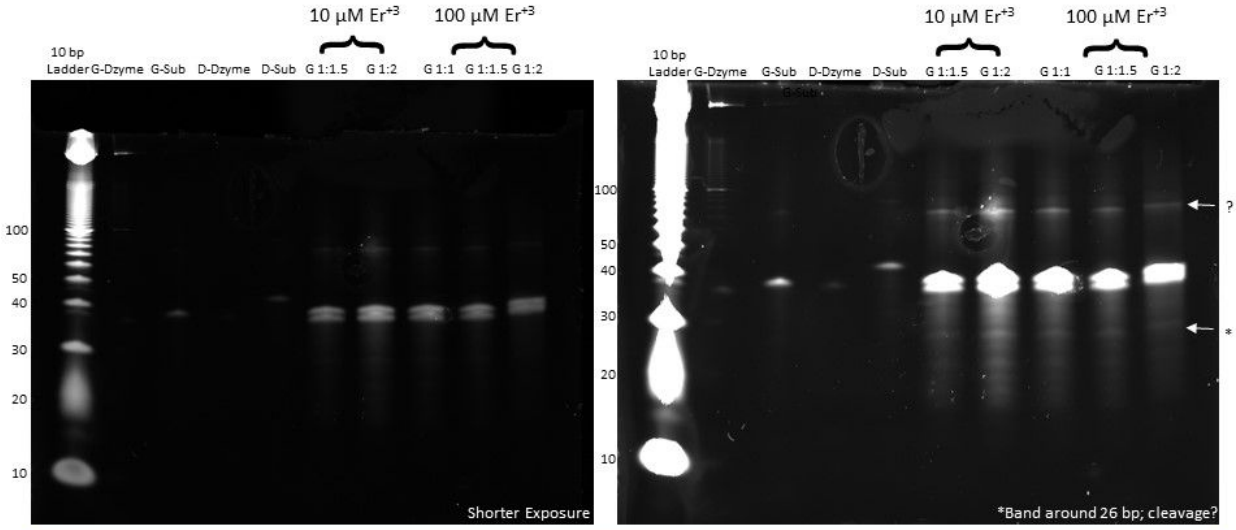
Load 10 ul of each reactions into gel

Load 10 ul of the ladder+buffer

Let it run for approximately 2.5 hours (or until it runs ¾ of the way down the gel)

Soak in SYBR Gold mixture for 30 minutes

15% Denaturing Gel_19 July 2016



Expected Sizes	
Collins G • PO Substrate: 40 bp • DNAzyme: 38 bp • After cleavage: 26 bp trigger + 14 bp	Collins D • PO Substrate: 46 bp • DNAzyme: 38 bp

Wednesday, July 20

Digest to Linearize more Terminator (Maya)

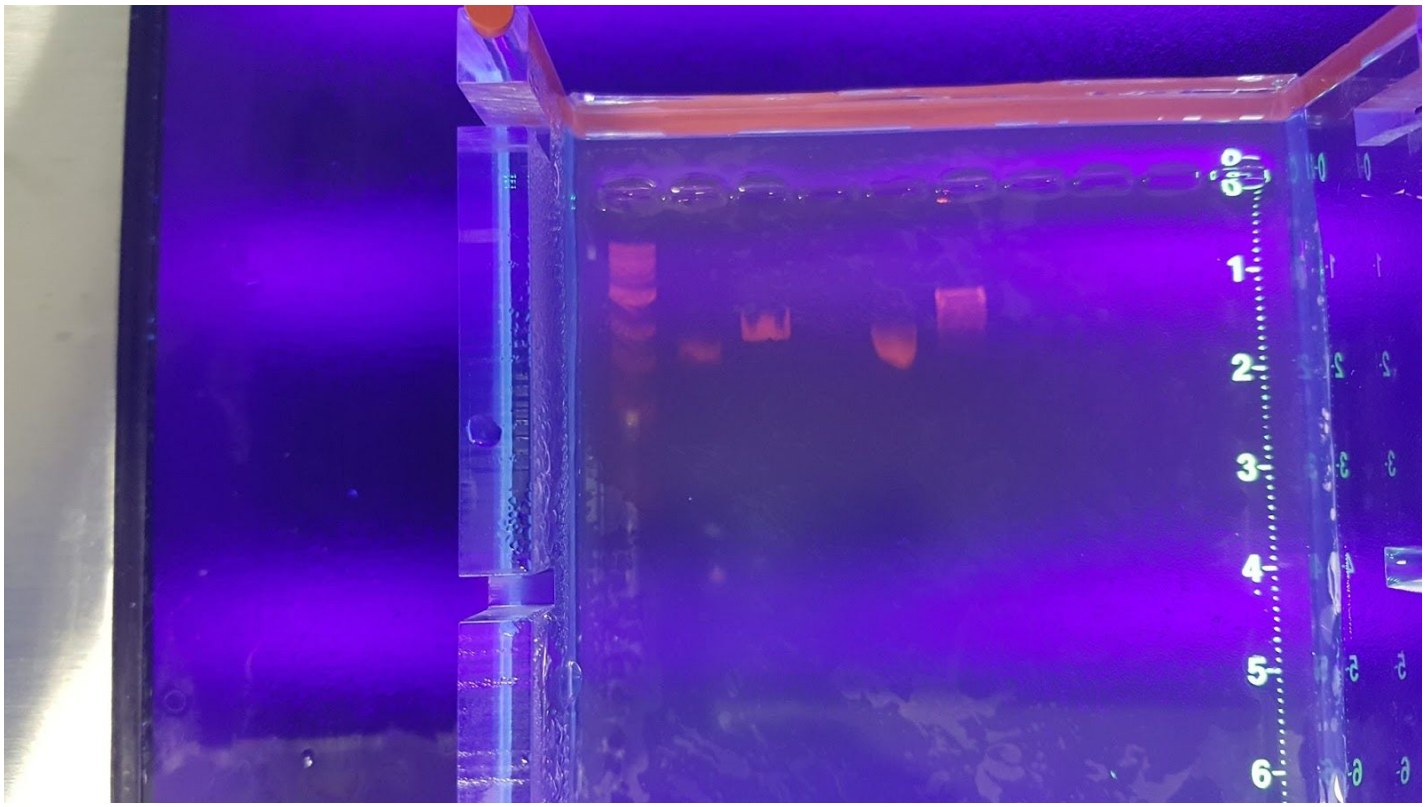
20 uL reactions with XbaI and EcoRI

1 uL of each enzyme
2 uL of buffer
1 ug plasmid
10.35 uL (96.9 ng/uL)
5.65 uL H2O

Gel for Terminators (Maya)

Checked other tubes that said Terminator

Lane 1: Ladder
Lane 2: Control (Uncut Term)
Lane 3: Linearized term
Lane 4: Term 1
Lane 5: Term 2
Lane 6: MLK term (looked digested)

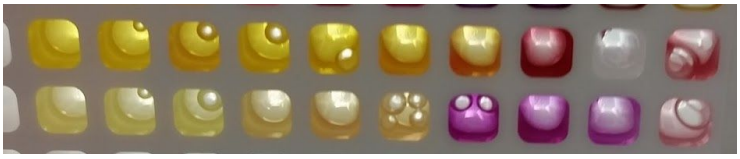


Decided to get rid of Term 1, Term 2, and MLK term since we did not know what they were

Liquid cultures for PT7-amilCP-Term, LacZ (?), and RBS-T3

Cell-free dilution 2 repeat with 2-hour incubation in tubes, not plate (Claire)

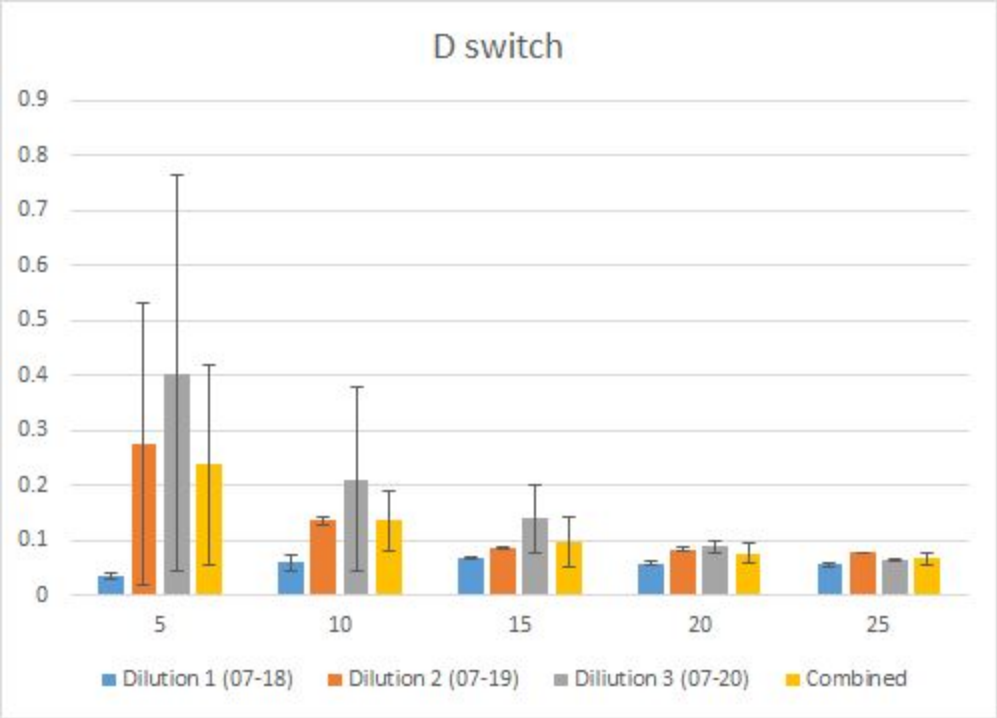
Row I: D switch
Row J: G switch
Columns 1-2: 5 uL
Columns 3-4: 10 uL
Columns 5-6: 15 uL
Columns 7-8: 20 uL
Columns 9-10: 15 uL



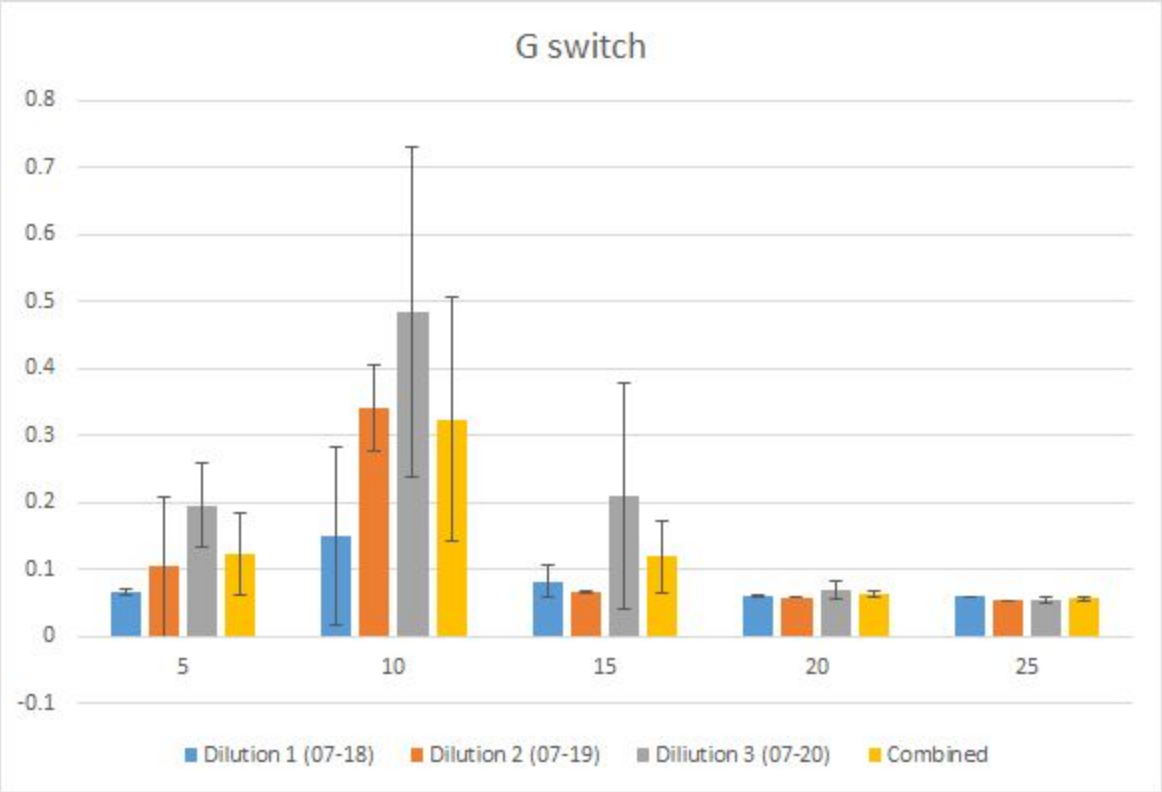
Overnight incubation of dilution 2

2-hour incubation results from all 3 dilutions:

D	Dilution 1 (07-18)				Dilution 2 (07-19)				Diliution 3 (07-20)				Combined	
Volume	Trial 1	Trial 2	Average	St Dev	Trial 1	Trial 2	Average	St Dev	Trial 1	Trial 2	Average	St Dev	Average	St Dev
5	0.0329	0.0385	0.0357	0.00396	0.0944	0.4557	0.27505	0.255478	0.6573	0.1495	0.4034	0.359069	0.23805	0.181031
10	0.049	0.0699	0.05945	0.014779	0.1418	0.131	0.1364	0.007637	0.3286	0.0941	0.21135	0.165817	0.135733	0.05573
15	0.0687	0.0673	0.068	0.00099	0.0837	0.0871	0.0854	0.002404	0.0968	0.1824	0.1396	0.060528	0.097667	0.046223
20	0.0605	0.0555	0.058	0.003536	0.0824	0.0861	0.08425	0.002616	0.0818	0.096	0.0889	0.010041	0.07705	0.017051
25	0.0575	0.0536	0.05555	0.002758	0.0787	0.0775	0.0781	0.000849	0.0674	0.065	0.0662	0.001697	0.066617	0.010418



G	Dilution 1 (07-18)				Dilution 2 (07-19)				Diliution 3 (07-20)				Combined	
Volume	Trial 1	Trial 2	Average	St Dev	Trial 1	Trial 2	Average	St Dev	Trial 1	Trial 2	Average	St Dev	Average	St Dev
5	0.0644	0.0698	0.0671	0.003818	0.1785	0.03006	0.10455	0.104581	0.2397	0.1523	0.196	0.061801	0.12255	0.061178
10	0.0555	0.2428	0.14915	0.132441	0.3856	0.2967	0.34115	0.062862	0.309	0.6572	0.4831	0.246215	0.324467	0.18222
15	0.0662	0.0995	0.08285	0.023547	0.0658	0.068	0.0669	0.001556	0.3299	0.0905	0.2102	0.169281	0.119983	0.054027
20	0.0608	0.0601	0.06045	0.000495	0.0588	0.0592	0.059	0.000283	0.0798	0.0606	0.0702	0.013576	0.063217	0.004267
25	0.0597	0.0604	0.06005	0.000495	0.0541	0.054	0.05405	7.07E-05	0.0525	0.0591	0.0558	0.004667	0.056633	0.002969



Transform D and G switches (Claire)

Time course of DNAzyme duplex reaction with erbium (Claire)

Sample at 0, 15, 30, 60, 120, 180, 360 minutes

Use D and G switches at 1:2 annealed ratio (10 uM : 20 uM)

Incubate at RT and 37 degC

10 uM erbium

4 conditions total (incubation temperature x switch)

50 uL reaction: 3.5 uL annealed duplex
0.5 uL 1 mM erbium
46 uL buffer B

At each time point, remove 6 uL reaction, quench with 6 uL loading buffer, freeze

Thursday, July 21

Camp BioE presentation (Praneeth and Maddie)

TECBio/DiSCoBio presentation (Claire, Maya, Aife)

Run 2 gels with Time course G DNAzyme reactions (Praneeth)

Load 10 ul of each reactions into gel

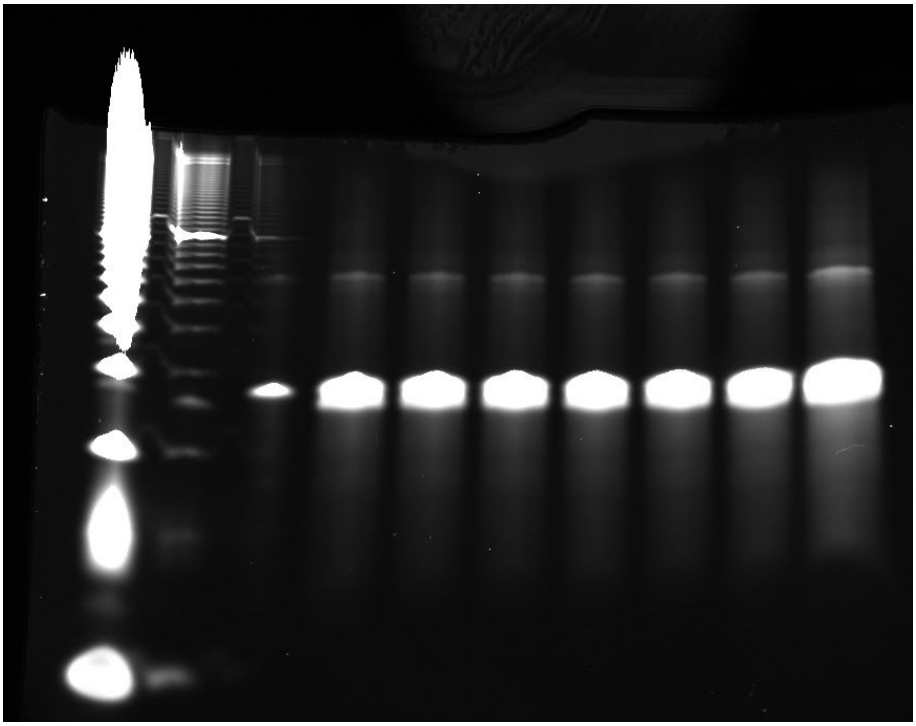
Load 10 ul of the ladder+buffer

Let it run for approximately 2.5 hours (or until it runs ¾ of the way down the gel)

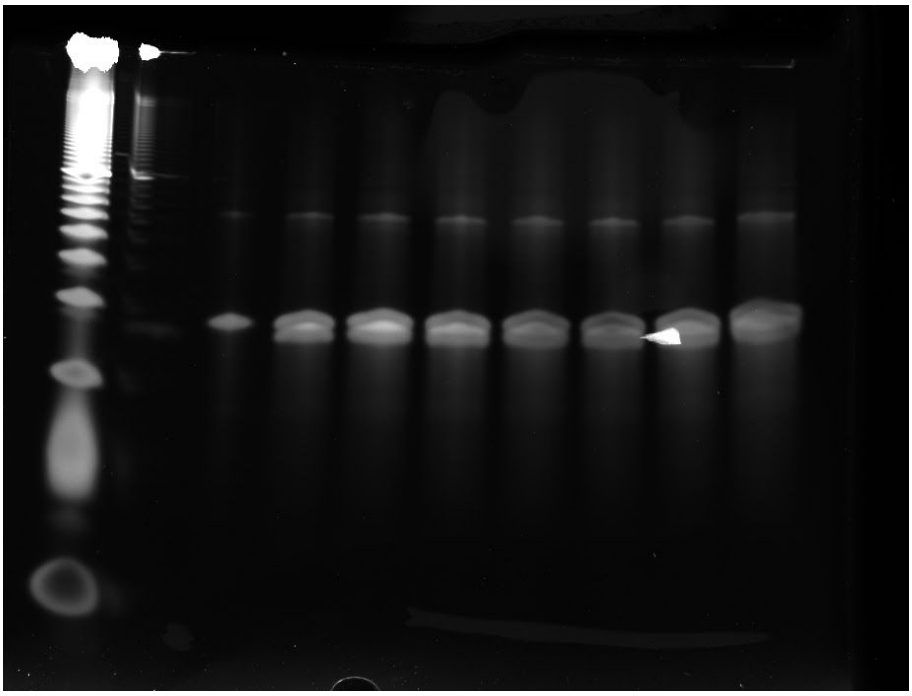
Soak in SYBR Gold mixture for 30 minutes

Lane	1	ladder	6	30 min
	2	P substrate	7	60 min
	3	DNAzyme	8	120 min
	4	0 min	9	180 min
	5	15 min	10	360 min

37 degrees:



Room temperature:



Mini-prep Ligation cultures (Maddie)

3:1 1: 166.7 ng/uL
2: 223.4 ng/uL
3: 138.6 ng/uL
4: 182.0 ng/uL
5: 32.6 ng/uL
6: 135.4 ng/uL
7: 144.7 ng/uL

7:1 None

BS-T3: 1: 173.5 ng/uL
2: 294.6 ng/uL

LacZ(?): 115.1 ng/uL

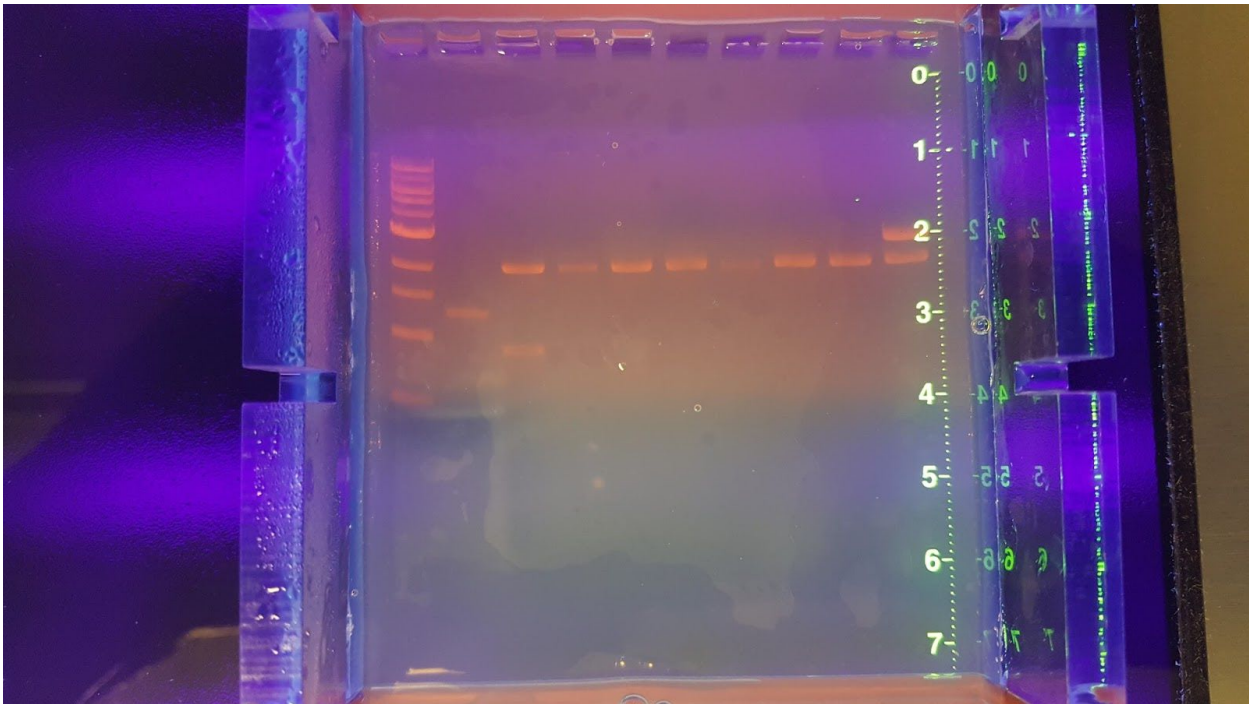
Digest of PT7-amilCP-Term and RBS-T3 (Maddie)

10 uL reactions

For each: 0.5 uL EcoRI
0.5 uL PstI
1 uL Buffer
1 uL plasmid (expect 3:1-5, 3uL)
7 uL H2O (expect 3:1-5, 5 uL)

Gel (Maddie/Maya)

Lane 1: Ladder
Lane 2: Control (Uncut Term)
Lane 3: 3:1-1
Lane 4: 3:1-2
Lane 5: 3:1-3
Lane 6: 3:1-4
Lane 7: 3:1-5
Lane 8: 3:1-6
Lane 9: 3:1-7
Lane 10: RBS-T3



Successful ligation with 3:1-1

Transformed into T7 cells

Gel confirmation of RBS-T3

Friday, July 22

Mid-Atlantic Meet-Up: Claire, Praneeth, and Maya at UMD

Miniprep Collins switches (Aife)

Most concentrated:	D	59.7 ng/uL
	G	84.1 ng/uL